Department of Defense
Legacy Resource Management Program

PROJECT NUMBER 12-635

PROJECT NAME/TITLE
Recovery Tool for Enhanced Black Abalone Recruitment on the California Channel Islands and Coastal Habitat

AUTHOR / ACTIVITY
Dr. David Lapota, SPAWAR Systems Center, Pacific

DATE OF REPORT October 2013

31 October 2013

INTERIM PROGRESS REPORT

"Recovery Tool for Enhanced Black Abalone Recruitment on the California Channel Islands and Coastal Habitat"

Project 12-635

By

David Lapota, Ph.D. and Melissa Blando, M.Sc

SUMMARY

In 2009, California (CA) State and Federal agencies declared the black abalone (*Haliotis cracherodii*), a marine gastropod mollusk, endangered under the Endangered Species Act. *H. cracherodii* ranges from northern CA to Baja CA, Mexico and occurs in rocky intertidal habitats to about 6 m depth. This accessibility exposes them to anthropogenic pressures from human harvesting, habitat destruction, and pollution more than other CA abalone species. In the wild, fertilization and recruitment rates of *H. cracherodii* are currently critically low due to the low density of reproductive adults, making it difficult for populations to recover from pressures such as Withering Foot Syndrome (WFS) and human harvesting (Chambers et al. 2006). Restoring wild populations therefore, is crucial, and the recovery of this endangered species may require human intervention. Specifically, optimal methods that enhance the production and settlement of larvae and recruitment of juveniles will be crucial in aiding its recovery. This may ultimately require rearing *H. cracherodii* juveniles in laboratory aquaculture settings and outplanting them into wild populations. However, little is known about how different adult rearing conditions will impact larval production and performance, or how these conditions impact the species' larval stages, survival and competency.

In this study, we will explore whether parental diet affects their fecundity, and how parental diet and larval rearing temperatures together affect growth, development, and settlement of their larval stages. Specifically, adult *H. cracherodii* have been fed diets that consist of either single species or mixed assemblages of different Phaeophyceaen (brown) and Rhodophyceaen (red) macroalgae, and their production of larvae quantified. Further, these larvae will be reared under different temperatures and their development and settlement will be monitored. Like other abalone species, *H. cracherodii* larvae are lecithotropic, indicating feeding does not occur during the larval stages. Rather, the larvae must rely on the yolk provided by the mother as an energy source, suggesting that parental condition may be crucial to larval settlement and competency, and ultimately to the successful recovery of the species. However, the variation in seawater temperature under which the larvae are released can also be important by either accelerating or delaying metamorphosis, suggesting this also may be important for their recovery. In fact, studies have shown differences in lipid and protein composition in *Haliotis* spp. larvae reared from parents fed varying diets, and temperatures under which their larvae were released significantly affect larval settlement and development. By investigating the potential synergistic effects of parental diet and seawater temperature on the development and settlement of *H. cracherodii* larvae we will identify optimal techniques for increasing larval production and settlement competency, and juvenile recruitment. In doing so, this study will support conservation efforts of this endangered species as well as provide possible methods for commercial farmers of other southern California *Haliotis* species.

INTRODUCTION

Southern California (CA) is home to seven abalone species in the genus *Haliotis*; *H. assimilis* (threaded), *H. corrugata* (pink), *H. cracherodii* (black), *H. fulgens* (green), *H. rufescens* (red), *H. sorenseni* (white), and *H. walallensis* (flat). Abalone are gastropod mollusks and follow a life history starting with male and female adults that broadcast spawn their gametes into the water column for eggs to be fertilized by sperm; then the first larval stage called the trocophore larval stage hatches out from a fertilized egg, (~22 hours after eggs have been fertilized); next the metamorphosis of the second larval stage called the veliger stage occurs (~43 hours after eggs have been fertilized); and lastly, settlement occurs where the post-larval/juvenile undergoes metamorphosis and sinks to the benthos (~68 hours after eggs have been fertilized), and grows into the adult stage (Jardillier et al. 2008). Abalone species inhabit coastal subtidal and intertidal habitats where they feed on various species of macroalgae.

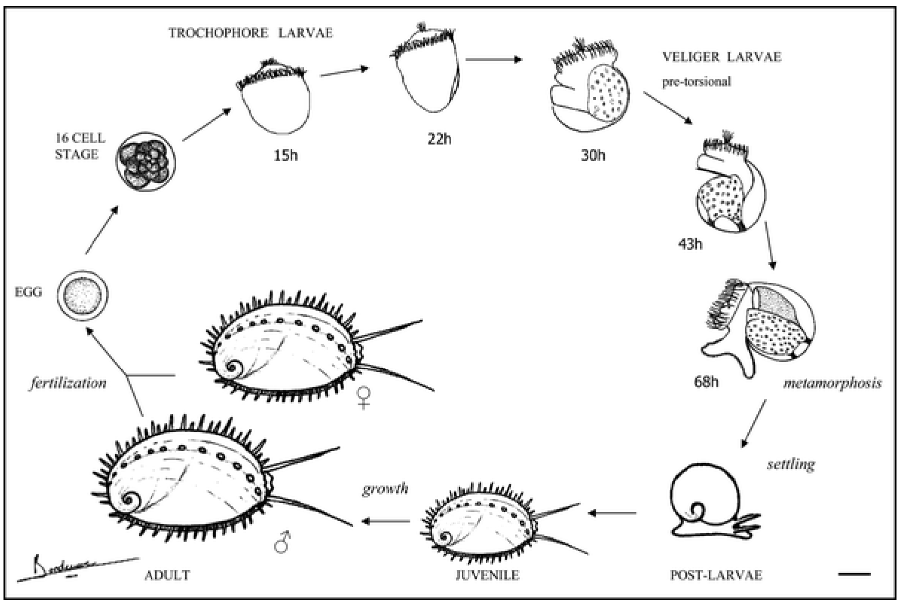
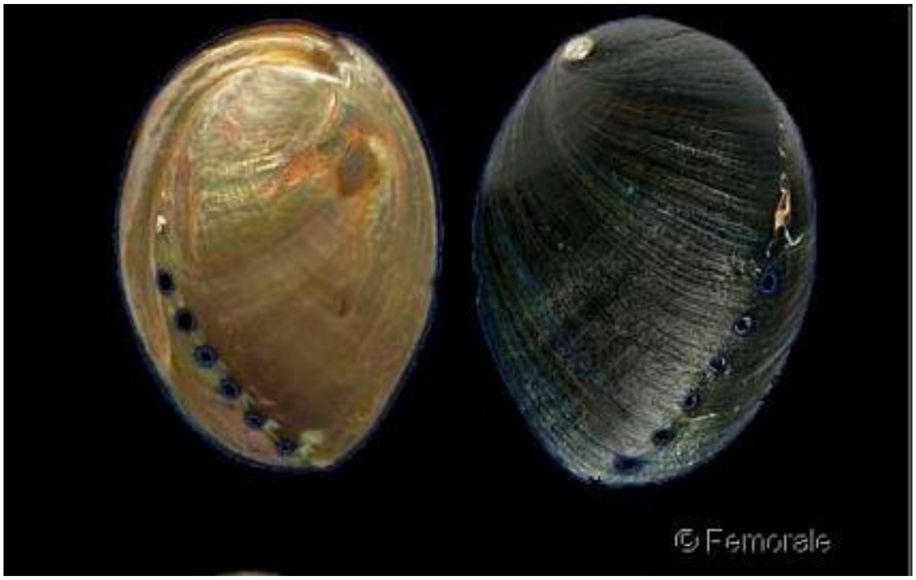


Figure 1. The life cycle of *Haliotis spp*. reveals the two yolk-bearing, free-swimming larval stages (trocophore and veliger) and settlement or juvenile/post-larval stage (from Jardillier et al. 2008)

Due to their feeding activity, they are often considered ecosystem engineers because they alter recruitment not only of their own species, but of other benthic invertebrates and algae as well (Matthews and Cook 1995; Miner et al. 2006). Furthermore, abalone are a highly prized commercial and recreational fishery species and comprise part of the diet for a number of invertebrate and vertebrate predators. However, due to human harvest and disease, their abundances in southern CA waters have declined over the past few decades, and many species are near the point where their populations are near extinction. Consequently, abalone have been protected from harvest, but their populations remain in dangerously low numbers. Therefore, activities to restore these populations in the wild are of paramount importance to the survival of these species and the recovery of the fisheries they once supported.

Currently, the Department of Defense (DoD) Legacy Resource Management Program funded a project interested in conditioning, spawning, settling, and growing out *H. cracherodii* for conservation purposes. Dr. David Lapota and Melissa Blando are working on this project to support the research goals of the program, in order to aquaculture *H. cracherodii* for future restoration outplants. Previously, we have worked with various techniques to increase numbers of juveniles (i.e. increase settlement) of other Haliotis species (*H. fulgens* and

H. rufescens) by utilizing artificial seawater, antibiotics, and the settlement cue γ-Aminobutryic Acid (GABA) to increase larvae survival and increase settlement. These techniques are widely utilized by commercial aquaculture farms, and the techniques are of interest to use on *H. cracherodii*. Specifically, we are interested in what factors enhance settlement of *H. cracherodii* larvae, such as parental diet and larval rearing temperature



.

Figure 2. Shell of the black Abalone, *Halitois cracherodii*

Previous research on *H. cracherodii* and other southern CA *Haliotis* species have examined the effects of parental diet and temperature on growth and food consumption. Specifically, Leighton and Boolootian (1963) observed that diets consisting of giant kelp, *Macrocystis pyrifera*, and/or the red macroalga, *Gigartina canaliculata*, produced higher growth rates in *H. cracherodii* adults than other types of macroalgae, though consumption rates of other macroalgal species such as of the featherboa kelp, *Egregia laevitaga* (*Egregia mensiezii*, Abbott and Hollenberg 1992), were greater. This suggests that there are differences in nutritional and/or energetic quality of their food. Another red macroalga, *Palmaria mollis*, increased growth rates in adult *H. rufescens*, and is considered an ideal food source in land-based aquaculture settings (Demetropoulos and Langdon 2004), though it remains unclear whether this species of macroalgae will produce similar growth in *H. cracherodii*. Similarly, mixed diets of brown and red algae have been shown to produce higher consumption and growth rates in abalone species around the world, especially when compared to monospecific diets of brown, red, or green algae Graham 2008; Qi et al. 2010). In New Zealand, for example, Stuart and Brown (1994) determined that a mixed diet of *M. pyrifera* and *Gracilaria chilensis* resulted in the highest growth rates of *H. iris* when compared to a mixed diet of *M. pyrifera* and *Ulva lactuca*. However, a mixed diet of *M. pyrifera* and *U. lactuca* is still of interest for *H. cracherodii,* especially since these are two common and easily accessible macroalgae in California. Thus, a primary focus of our research will be to identify different mixtures of macroalgal species as potential food sources for *H. cracherodii* aquaculture. Diet is crucial in determining how to condition *H. cracherodii*, adults, because diet can promote weight gain, which may increase spawning success.

Figure 3. Baseline information for the black abalone. (Photos by Blando and VanBlaricom).

In addition to adult food, the temperature at which the adults and their larvae are grown can also be important to the growth and reproduction of laboratory-reared abalone. For example, Larvae of three *Haliotis* species reared at temperatures ranging from 15 -23°C grew more rapidly and exhibited higher settlement compared to larvae reared at temperatures outside of this range (Leighton 1974). Further, Vandepeer (2006) emphasized the interaction between nutrition and temperature as they impact mortality of *H. rubra*, suggesting interactions between adult diet and larval rearing temperature may also be of great importance for *H. cracherodii*. Likewise, Nelson et al. (2002) linked the effect of parental diet on larval lipid concentrations in *H. fulgens*. Specifically, they demonstrated that a parental diet of *E. menziesii* resulted in the greatest preference by, and growth in, adult *H. fulgens*. They also determined that larvae from parents fed *E. menziesii* contained the greatest lipid content of polyunsaturated fatty acids, which was the highest represented lipid class in *H. fulgens* larvae, and concluded selective storage of lipids in adult tissues, and subsequent transfer to the larvae. While these studies have all considered the effects of diet and/or temperature on a variety of abalone species, no studies of which I am aware have identified optimal temperatures for larval development and settlement in *H. cracherodii*, and parental diet has not been clearly linked to temperature rearing effects on their larvae. This information may prove critical in the successful restoration of black abalone populations.

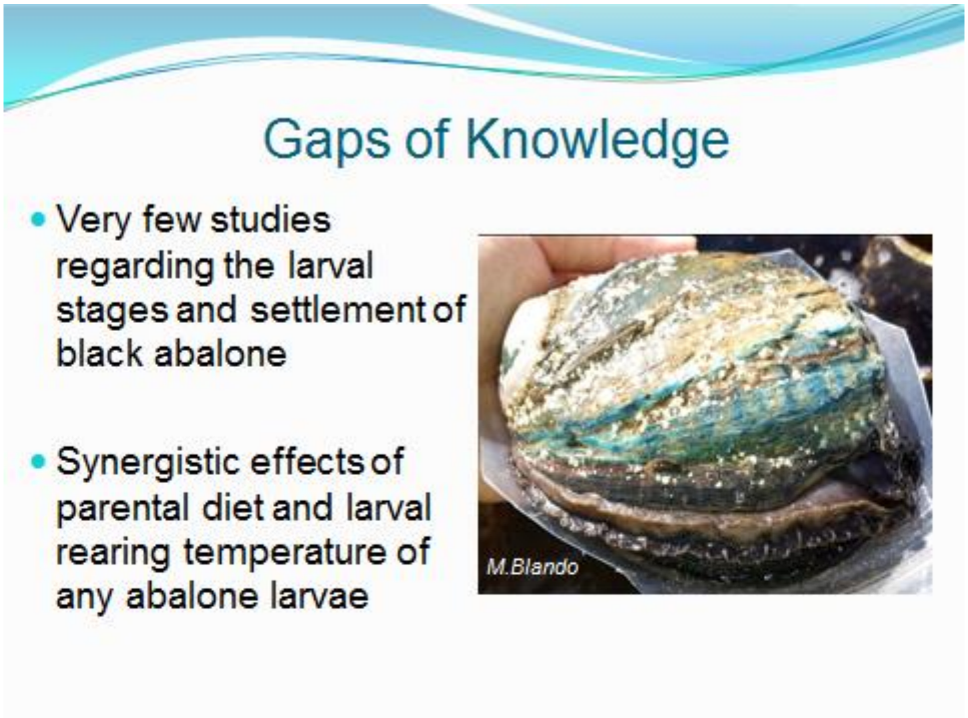


Figure 4. Limited data quantifying proposed rearing of black abalone larval stages.

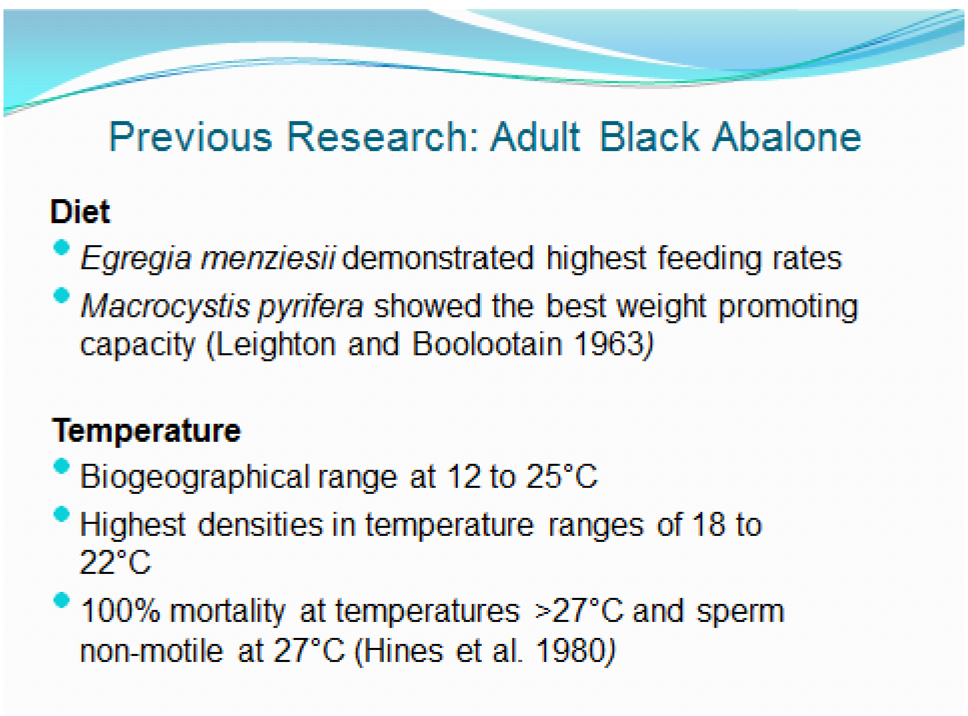


Figure 5. Earlier data addressed diet and culture temperature for adult black abalone for limited periods of time for adult black abalone, but not for larval stages.

CURRENT PRELIMINARY RESEARCH

Inducing spawning in *H. cracherodii* has been historically difficult (pers. comm. Dr. Caroline Freedman ,2013). Additionally, the lack of research on *H. cracherodii* settlement techniques leads to uncertainty. It is crucial for us to conduct preliminary research utilizing *H. fulgens* as a surrogate species to determine the appropriate way to induce spawning of adults, and combination of seawater type, antibiotic type and concentration, food type, and settlement cue type and concentration for larvae. Furthermore, once we determine these parameters that induce spawning and increase settlement using *H. fulgens*, the methods will be applied to *H. cracherodii*.

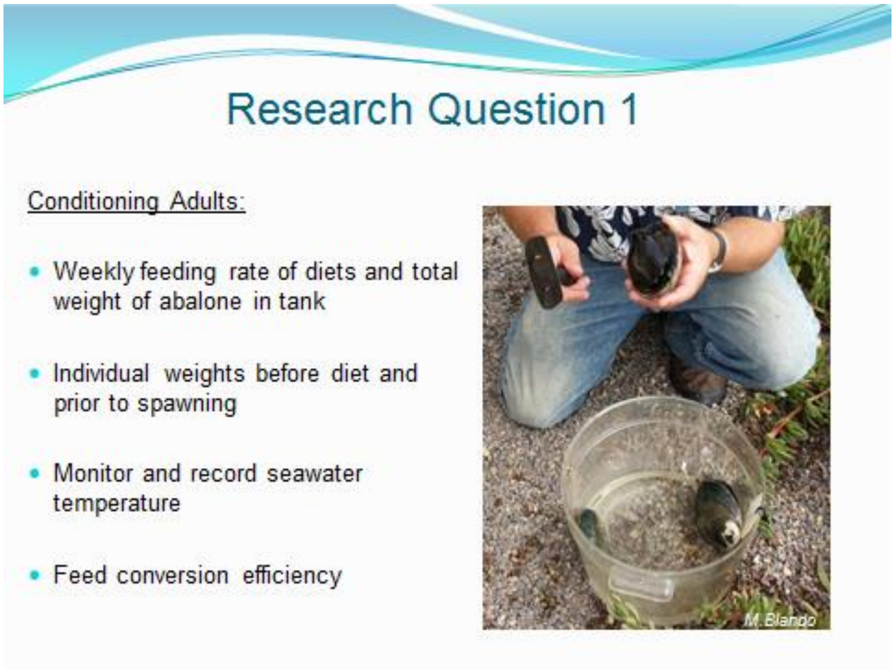


Figure 6. The conditioning of the adult black abalone had to be initially addressed.

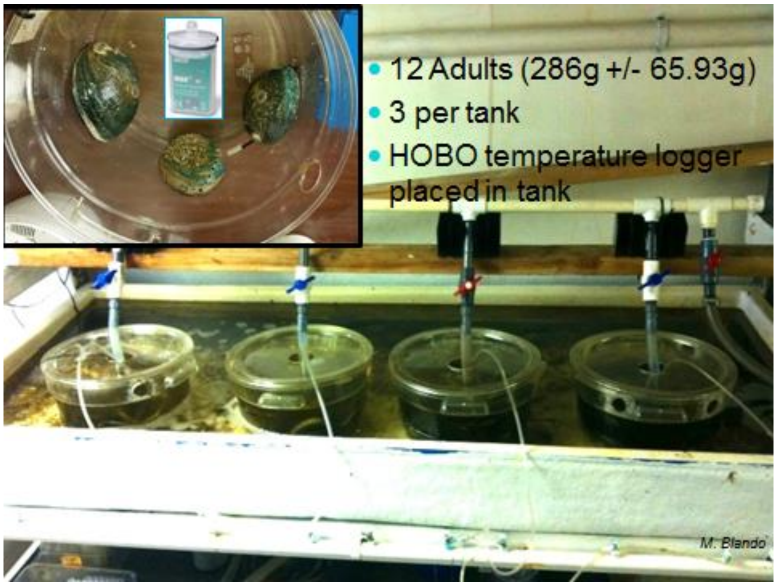


Figure 8. Laboratory culture setup for feeding the adults. All containers received running seawater. (Photos by Blando).

Figure 9. Algal diets were chosen to condition the adult black abalone. Various combinations of algae and algal quantities were added to each of the 4 containers containing the adults.

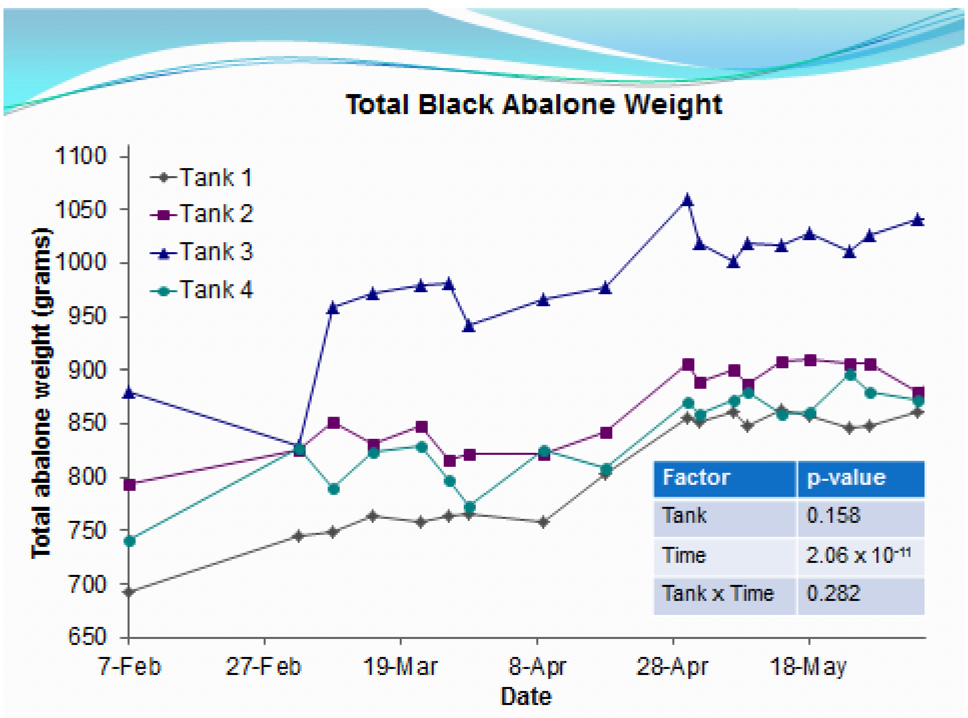


Figure 10. Accumulative weight gain (in grams) in adults for each of the 4 culture containers over time. All abalone showed a positive increase in weight.

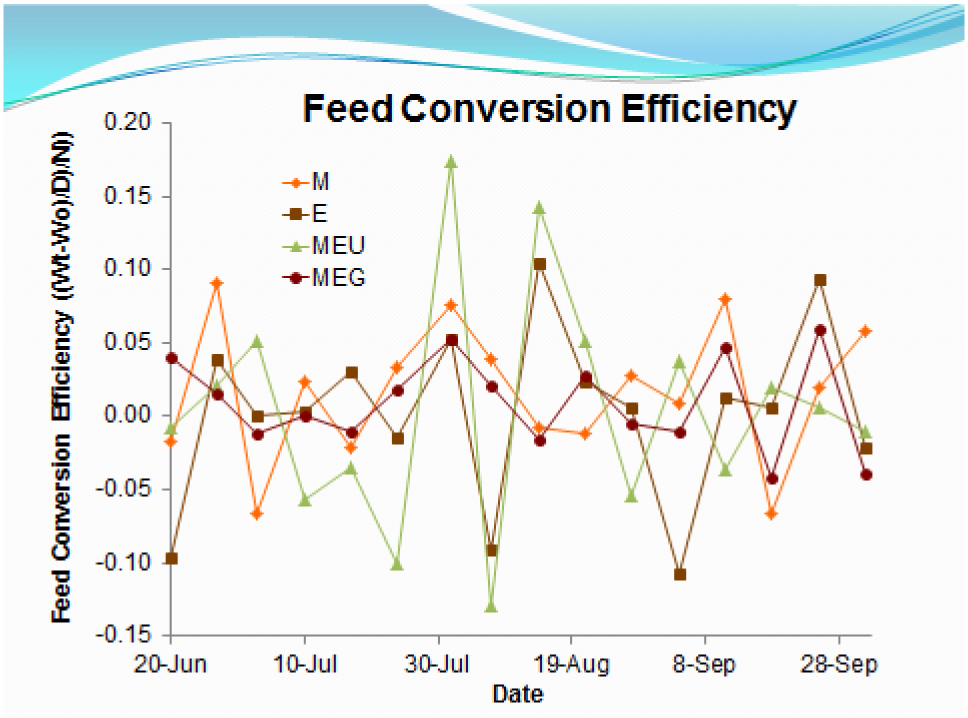


Figure 11. All diets showed a high variation in feeding conversion efficiency (amount of algae consumed to net increase in weight in the adults. This variation may be attributed to seawater temperature and the quality (nutrition) of the algae consumed.

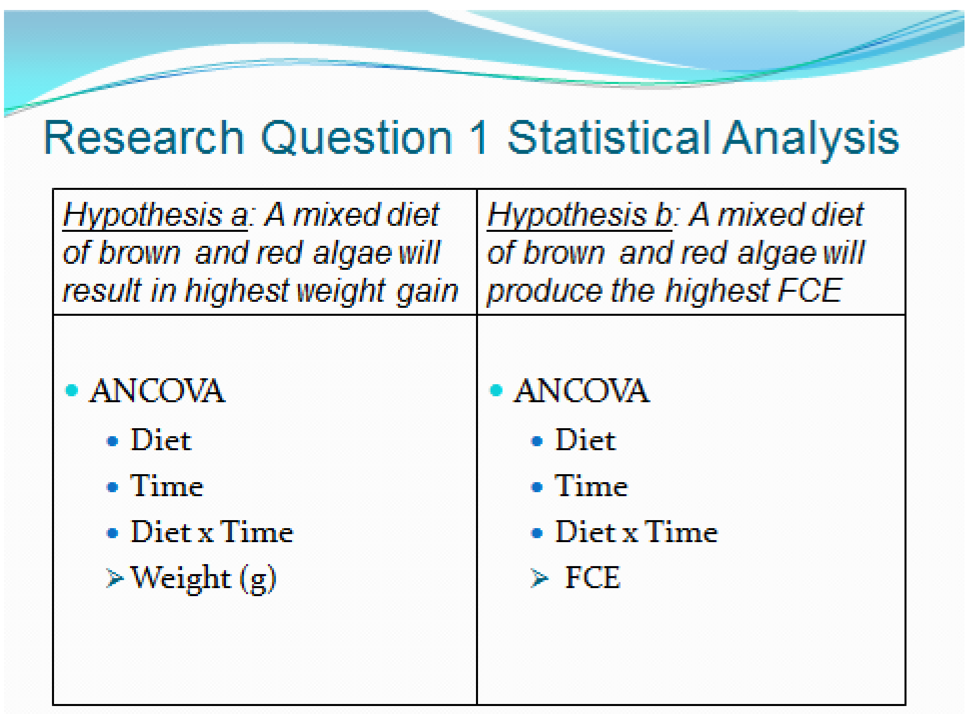


Figure 12. Statistically, we tested 2 hypothesis which compare the effect of mixed diets on weight gain and the highest food conversion efficiency (FCE).

Successful spawning of *H. fulgens* by exposure to hydrogen peroxide and sodium hydroxide was completed at SSC Pacific Abalone Farm in June - July 2013.

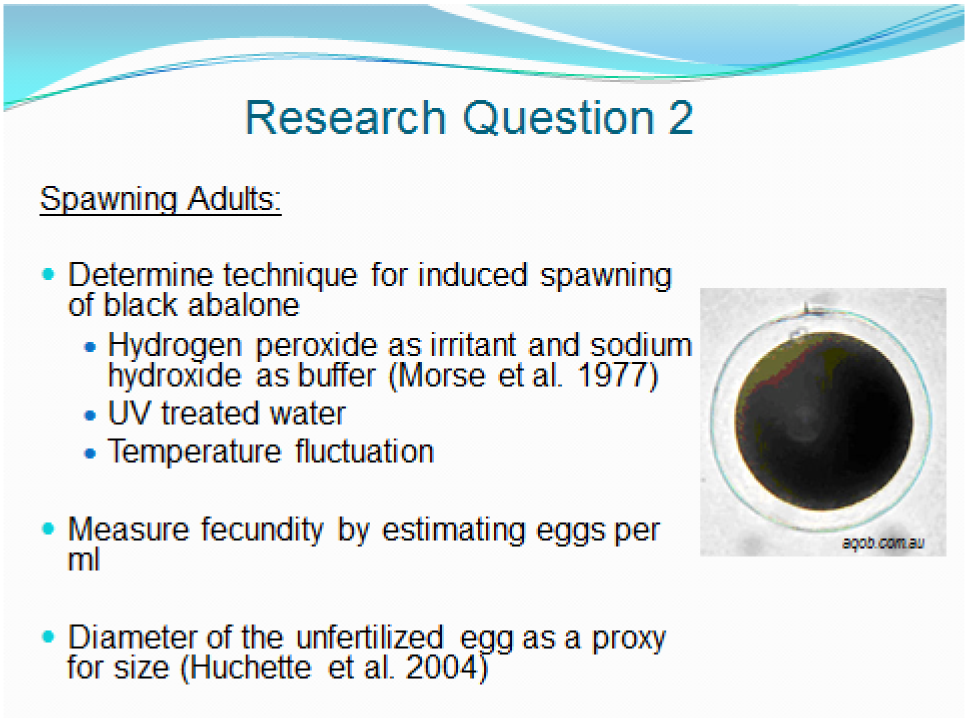


Figure 13. All 3 methods for spawning induction have been successful for the surrogate species *Haliotis rufescens* and *H. fulgens*. Because UV induction was very successful with both conditioned red and green abalone, this is our method of choice for future spawning in the black abalone.

In order to increase settlement success, we conducted two experiments to test the effects of: (1) seawater type, (2) antibiotic type and concentration, and (3) food type and settlement cue type and concentration. Specifically, we tested three factors as they impact settlement of *H. fulgens*: (1) seawater type; artificial vs. 60 µM filtered seawater, (2) antibiotic type; gentamicin vs. kanamycin, and (3) antibiotic concentration; low, medium, and high. The concentrations for kanamycin were: low (0.175 mg/L), medium (0.349 mg/L), and high (0.698 mg/L). Additionally, the concentrations for gentamicin were: low (0.261 mg/L), medium (0.522 mg/L), and high (1.04 mg/L). There was no settlement of *H. fulgens* within the filtered seawater treatment, which suggested that artificial sweater in a static renewal system will be used for rearing larvae. Subsequently, only the data for treatments of artificial seawater were analyzed. There was a significant interaction of antibiotic type and concentration. In artificial seawater, a low concentration of gentamicin demonstrated significantly higher settlement compared to high or medium concentrations of gentamicin or high, medium and low concentrations of kanamycin. Antibiotics were only used for two days after egg hatching, and all treatments were induced to settle utilizing GABA (Strathmann 1987). In experiment (2), we tested food type because once larvae have metamorphosed and settled, they begin to feed on diatom films.

Figure 14. Experimental setup for spawning the black abalone. Abalone were placed singly into individual containers. Ejected gametes (sperm and eggs) would be collected and fertilized with other gametes from reared abalone fed unique diets. Because of the limited spawning period of the black abalone (Fall, usually), we were not able to use UV induction. The hydrogen peroxide stimulus was used once and was not successful. This experiment will be conducted again in the summer-fall time frame in 2014.

Additionally, GABA concentration is of interest to determine the amount of the cue the larvae need to settle. We tested two factors: (1) food type: *Navicula* (a diatom species) vs. Phytofeast (a commercial feed) and (2) GABA concentration; low, medium, high, and crustose coralline algae (CCA) rock. The GABA concentrations were: low (0.5 mg/L), medium (1 mg/L), and high (2 mg/L). The crustose coralline rock emitted an unknown natural concentration. A two-way analysis of variance determined that both food type and settlement type were significant, and there was no interaction between them. A low concentration of GABA and a diet of *Navicula* was determined to have a 20% settlement success of juvenile *Haliotis fulgens*, which was significantly higher than treatments containing Phytofeast, high and medium concentrations of GABA, and crustose coralline algae rocks.

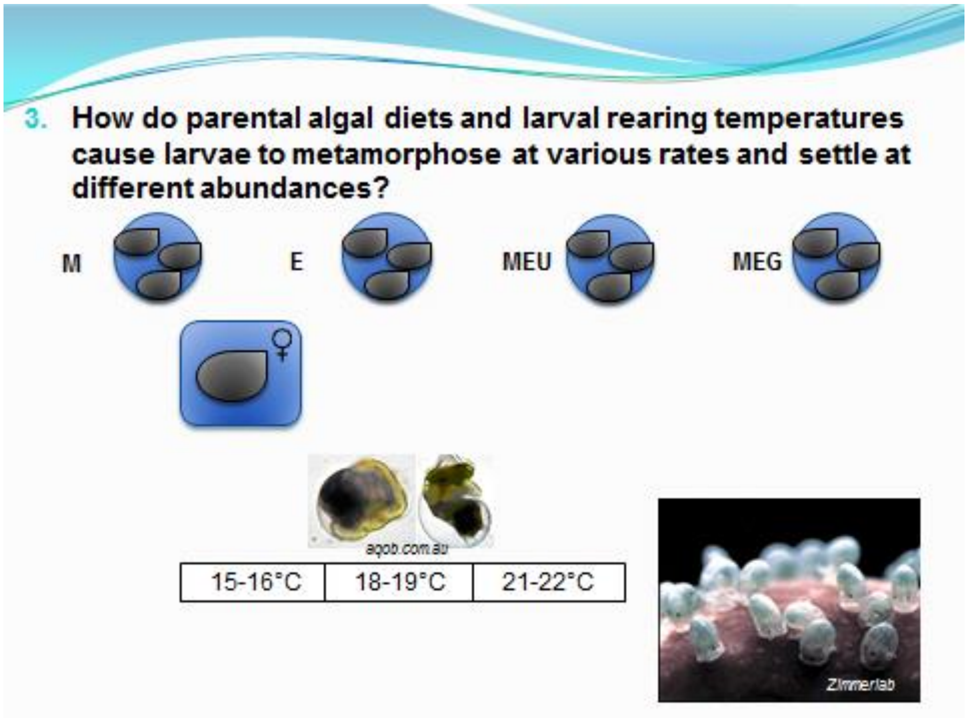


Figure 15. Research question on the relationship of what effect do parental diets and rearing temperatures have on successful settling of the larvae. This question was not answered due to limitations of the time required to fully condition the adult black abalone. This aspect will be addressed during the summer 2014.

In previous studies, crustose coralline algae have been found to induce settlement of *Haliotis* species (Morse et al. 1977). However, during our experiment, crustose coralline algae rocks introduced predators of *H. fulgens* juveniles. Crabs and worms were observed feeding on shells of both the veliger stage and juvenile *H. fulgens*. The two experiments, utilizing *H. fulgens* as a surrogate species, suggest that for this study, we should use artificial seawater, a low concentration of gentamicin, and a low concentration of GABA to induce settlement of *H. cracherodii,* and *Navicula spp*. as a post-larval/juvenile food type.

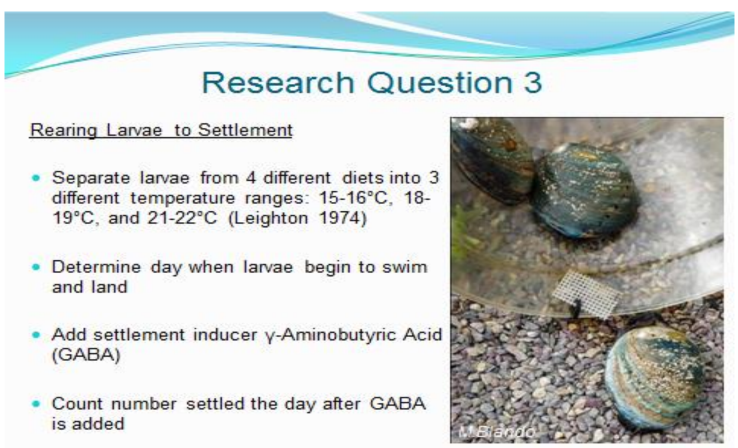


Figure 16. Protocol method to address successful settlement of black abalone larvae. This will be tested in the summer 2014.

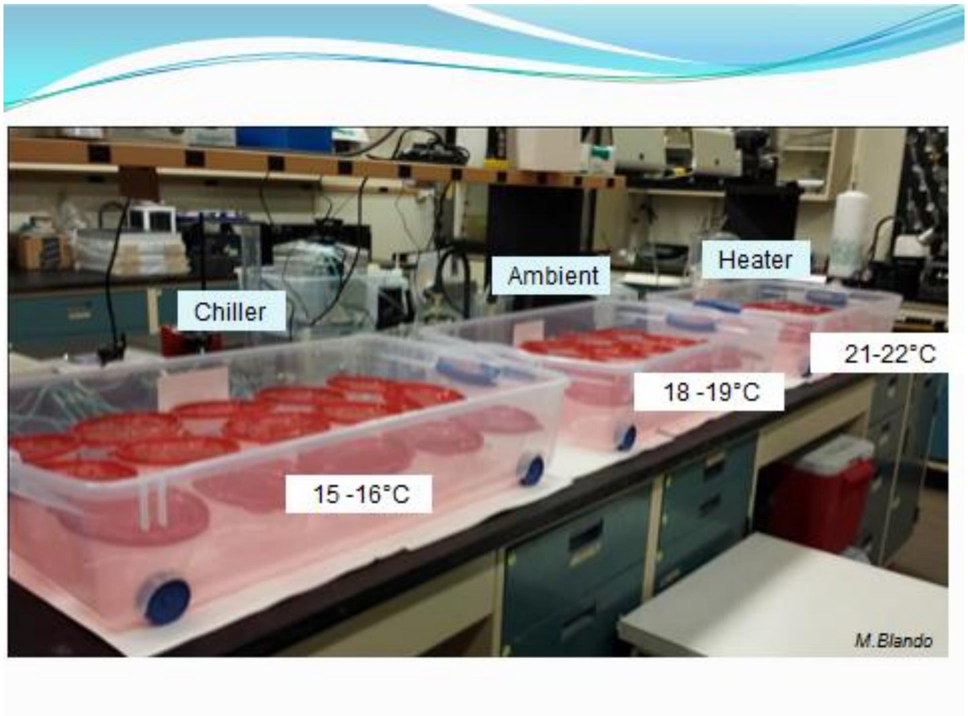


Figure 17. Laboratory set up for settling abalone larve at 3 temperature ranges. This will address aspects listed in question 3 (above). The system is a static renewal system where diatom diets will be provide at set temperature ranges. These ranges overlap those temperatures encountered by wild larvae.

It is essential to refine settlement techniques using a different *Haliotis* species, prior to handling *H. cracherodii* larvae. This will address aspects listed in question 3 (above). The system is a static renewal system where diatom diets will be provide at set temperature ranges. These ranges overlap those temperatures encountered by wild larvae.

SIGNIFICANCE

Improving laboratory spawning and culturing techniques of various southern CA abalone species in order to increase larval settlement and competency is crucial in supporting conservation efforts and commercial abalone farms. By increasing larval settlement and juvenile recruitment in aquaculture settings, more abalone will be available to outplant into the wild populations, thereby enhancing the potential success of recovery activities. The results of thorough experimentation will help various institutions understand how to successfully spawn and culture southern CA abalone species, and these techniques will provide vital information in an attempt to aid in their recovery. In addition to improving spawning and settlement techniques, outplanting is crucial in restoring natural populations (Lapota et al. 2000). Outplanting seeds natural populations with laboratory reared propagules, which increases the number of reproductive individuals in the population and may increase the number of eggs and sperm in the water column during aggregated spawning events. This will increase genetic diversity of the wild population and will assist in the recovery of this ecologically and commercially important species. Results from this study may also be used to aid in restoring other depleted abalone populations such as the white abalone, *H. sorenseni*. These species are the first two marine invertebrates to be listed as endangered, and more *Haliotis* species will likely follow this trend if nothing is done. More research of this nature is desperately needed to conserve and restore wild populations of all southern CA abalone. Furthermore, additional research is needed on how to successfully spawn southern CA abalone species in the laboratory, increase settlement of laboratory-reared juveniles, assess the success of outplanted juveniles, and in overcoming the effects of withering foot syndrome.

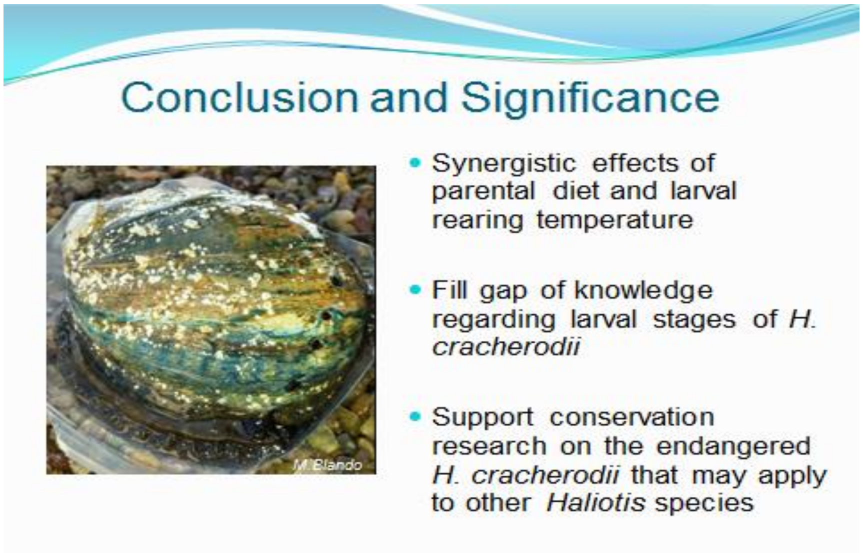


Figure 18. Gaps that remain for successful spawning of the black abalone.

CONCLUSIONS

While all aspects of this study were not completely addressed in FY12,we do anticipate completion of the remaining research questions in the summer 2014. We have investigated diets which show promise for conditioning adult abalone and have observed seawater temperature controls which affect weight gain and weight loss in the abalone. Temperature is critical for satisfactory development of gamete production. Cooler temperatures appear to be less stressful and encourage an increase in body weight and gonad development. Higher seawater temperatures appear to be more stressful as body weights leveled off and even decreased with sustained elevated temperatures. We have also observed that no one diet is conducive to maximum weight gain, however, mixed diets (consisting of red, green, and brown algae) promoted a greater feeding rate and increased gonad development. Identification to sex was observable toward the end of the summer 2013. Only one spawning attempt was conducted with hydrogen peroxide which proved to be unsuccessful. By continuing to condition the abalone from December 2013 to April 2014, we have observed a positive weight gain and have observed an interesting trend of weight gain, weight loss, with decreased water temperatures and increased water temperatures, respectively. By manipulating these variables and a mixture of spawning methods (temperature shock, hydrogen peroxide, and use of ultra-violet light stimulation), spawning activities will allow us to pursue and answer the remaining research questions.

ACKNOWLEDGEMENTS

This work, Project 12-635, was funded by the DoD Legacy Resource Management Program and is gratefully acknowledged.

LITERATURE CITED /RELATED REFERENCES

Abbott, I.A. and G.J. Hollenberg. 1992. Marine Algae of California. Stanford University Press, Stanford, California, USA

Ault, J.S. 1985. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Southwest) black, green, and red abalones. Department of the Interior U.S. Fish and Wildlife Service, Washington, DC, United States of America.

Butler, J., A. DeVogelaere, R. Gustafson, C. Mobley, M. Neuman, D. Richards, S. Rumsey, B. Taylor, and G. VanBlaricom. 2009. Status Review Report for Black Abalone (Haliotis cracherodii Leach, 1814). NOAA's National Marine Fisheries Service (NMFS), Long Beach, CA, United States of America.

Chambers, M. D., G. R. VanBlaricom, L. Hauser, F. Utter, and C. S. Friedman. 2006. Genetic structure of black abalone (Haliotis cracherodii) populations in the CA islands and central CA coast: Impacts of larval dispersal and decimation from withering syndrome. Journal of Experimental Marine Biology and Ecology 331:173-185.

Demetropoulos, C. and C. Langdon. 2004. Pacific dulse (Palmaria mollis) as a food and biofilter in recirculated, land-based abalone culture systems. Aquacultural Engineering.32:57-75

Gardner, G.R., J.C. Harshbarger, J.L. Lake, T.K. Sawyer, K.L. Price, M.D. Stephenson, P.L. Haaker, and H.A.Togstad. 1995. Association of prokaryotes with symptomatic appearance of withering syndrome in black abalone Haliotis cracherodii. Journal of Invertebrate Pathology.66:111-120

Graham, M.H. 2008. Integrated culture of seaweeds and red abalone in Monterey Harbor. UC San Diego: California Sea Grant College Program. Retrieved from: http://www.escholarship.org/uc/item/7m8291kw

Heasman, M. and N. Savva. 2007. Manual for intensive hatchery production of abalone. NSW Department of Primary Industries, Taylors Beach, NSW, Australia.

Hines, A., Anderson, S. AND Brisbin, M. (1980). Heat tolerance in the black abalone Haliotis cracherodii: Effects of temperature fluctuation and acclimation. Veliger 23, 113-118.

Huchette, S.M.H, J.P. Soulard, C.S. Koh, and R.W. Day. 2004. Maternal variability in the blacklip abalone, Haliotis rubra leach (Mollusca: Gastropoda): effect of egg size on fertilisation success. Aquaculture 231:181-195

Jardillier, E., M. Rousseau, A. Gendron-Badou, F. Fröhlich, D. C. Smith, M. Martin, M. N. Helléouet, S. Huchette, D. Doumenc and S. Auzoux-Bordenave. 2008. A morphological and structural study of the larval shell from the abalone Haliotis tuberculata. Marine Biology. 154: 735-744

Kingsley-Smith, P.R., C.A. Richardson, and R. Seed. 2005. Growth and development of the veliger larvae and juveniles of Polinices pulchellus (Gastropoda: Naticidae). J. Mar. Ass. U.K. 85:171-174

Lapota, D., R. Gunther, J. Chock, and C.H. Liu. 2000. Red and green abalone seed growout for reseeding activities off Point Loma, California. Journal of Shellfish Research 19:431-438

Leighton, D., and R. A. Boolootian. 1963. Diet and growth in the black abalone, Haliotis cracherodii. Ecology. 44:227-238.

Leighton, D. 1974. The Influence of temperature on larval and juvenile growth in three species of southern CA abalone. Fishery Bulletin. 72:1137-1145

Litaay, M. and S.S. De Silva. 2003. Spawning season, fecundity and proximate composition of the gonads of wild-caught blacklip abalone (Haliotis rubra) from Port Fairy waters, south eastern Australia. Aquatic Living Resources. 16:353-361

Matthews I, Cook P.A. 1995. Diatom diet of abalone post- larvae (Haliotis midae) and the effect of pre-grazing the diatom overstorey. Marine and Freshwater Research 46:545-548

Mccormick, T.B., L.M. Buckley, G. Navas, G. Barber, B. Billups, V. Gill, B. Jones, N. Peterson, B. Saylor, and J. Sayre. 2012. Larval competency of red abalone (*Haliotis rufescens*): A new timeframe for larval distribution. Journal of Shellfish Research 31:1183-1187.

Miner, C. M., J. M. Altstatt, P. T. Raimondi, and T. E. Minchinton. 2006. Recruitment failure and shifts in community structure following mass mortality limit recovery prospects of black abalone. Marine Ecology Progress Series 327:107-117.

Morse, D. E., H. Duncan, N. Hooker, and A. Morse. 1977. Hydrogen peroxide induces spawning in mollusks, with activation of prostaglandin endoperoxide synthetase. Science. 196: 298-300

Nelson, M.M., D.L. Leighton, C.F. Phleger, and P.D. Nichols. 2002. Comparison of growth and lipid composition in the green abalone, Haliotis fulgens, provided specific macroalgal diets. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 131:695-712

Qi, Z.H., H.M. Liu, B. Li, Y.Z. Mao, Z.J. Jiang, J.H. Zhang, J.G. Fang. 2010. Suitability of two seaweeds, Gracilaria lemaneiformis and Sargassum pallidum, as feed for the abalone Haliotis discus hannai ino. Aquaculture 300:189–193.

Shallow Seafarming Research Institute in Kosong. 1990. Training manual on artificial breeding of abalone (*Haliotis discus hannai*) in Korea DPR. Fisheries and Aquaculture Department, Democratic People’s Republic of Korea. Retrieved from: http://www.fao.org/docrep/field/003/ab731e/AB731E00.htm#TOC

Strathmann, M.F. 1987. Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast. University of Washington Press, Seattle, Washington, United States of America.

Vandepeer, M. 2006. Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature. SARDI Aquatic Sciences. RD02/0035-2. Australian Government. Fisheries Research and Development Corporation. Adelaide, South Australia, Australia.

Vavra, J. and D. Manahan. 1999. Protein Metabolism in Lecithotrophic Larvae (Gastropoda: Haliotis rufescens). Biological Bulletin. 196:177-186

Vilchis, I. L., M.J. Tegner, J.D. Moore, C.S. Friedman, K.L. Riser, T.T. Robbins, and P.K. Dayton. 2005. Ocean warming effects on growth, reproduction, and survivorship of southern California abalone. Ecological Applications 15:469-480.